Reducing Animal Use for Acute Oral Toxicity Testing: ICCVAM Recommendations for the Use of *In Vitro* Cytotoxicity Test Methods.

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Animal use for *in vivo* acute oral toxicity tests (i.e., the Up-and-Down Procedure [UDP] and the Acute Toxic Class [ATC] method) may be reduced by using data from *in vitro* cytotoxicity methods to estimate starting doses for the studies. ICCVAM evaluated two *in vitro* neutral red uptake (NRU) basal cytotoxicity test methods (BALB/c murine fibroblasts [3T3] and primary normal human epidermal keratinocytes [NHK]) and published recommendations on their usefulness and limitations. The *in vitro* data were used to estimate the LD_{50} of up to 68 substances, and animal savings were calculated using starting doses based on these LD_{50} estimates. The *in vitro* methods would reduce animal use by 5-28%, (0.5-3.3 animals per test) with savings greatest for chemicals with low toxicity. The *in vitro* methods, which correctly predicted the UN Globally Harmonized System (GHS) acute oral toxicity category for only 29-31% of the substances, are not sufficiently accurate for hazard classification. However, ICCVAM recommends that the NRU test methods be considered in a weight-of-evidence approach to estimate starting doses for acute oral toxicity tests and that these methods be considered before animal tests are conducted. Supported by NIEHS contract N01-ES 35504.